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Increased CYP2J Expression and Epoxyeicosatrienoic Acid Formation in Spontaneously Hypertensive Rat Kidney

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ABSTRACT

Epoxyeicosatrienoic acids (EETs) are major products of cytochrome P450 (CYP)-catalyzed metabolism of arachidonic acid in the kidney. The potent effect of EETs on renal vascular tone and tubular ion and water transport implicates their role in the regulation of renal function and blood pressure. The present study was designed to test the hypothesis that CYP-catalyzed EET formation was altered in the spontaneously hypertensive rat (SHR) kidney. The formation of 14,15- and 11,12-EET was ~2-fold higher in incubations of arachidonic acid with SHR renal cortical microsomes relative to microsomes from normotensive Wistar-Kyoto (WKY) rats. This was consistent with increased expression of a CYP2J2 immunoreactive protein in the SHR cortex and outer medulla. In contrast, there was no significant difference in the levels of the CYP2E and CYP2C epoxygenases in SHR and WKY kidneys. Protein and RNA analysis suggests that the CYP2J2 immunoreactive protein that is overexpressed in the SHR kidney is distinct from the known rat CYP2J isoforms. EET formation also was documented in vivo from measurements of urinary EET excretion. Importantly, the excretion rates of 14,15-, and 11,12-EETs were 2.5- and 1.8-fold higher, respectively, in SHR than WKY kidney. These studies provide both in vitro and in vivo evidence for increased EET formation in the SHR kidney and identify a novel CYP2J2 immunoreactive protein that is differentially expressed in the hypertensive kidney. In light of the known biological properties of the EETs, these findings may be important in elucidating the mechanisms that control renal vascular tone and tubular ion transport in the SHR.

Arachidonic acid is a major component of the membrane phospholipid pool and an important precursor of numerous eicosanoids. Although metabolism of arachidonic acid by cyclooxygenases and lipoxygenases has been well characterized, the cytochrome P450 (CYP) monooxygenases have only recently been recognized for their important role in eicosanoid formation. The major products of CYP-catalyzed arachidonic acid metabolism are regio- and stereoisomeric epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE) (Makita et al., 1996). The EETs are further metabolized by soluble epoxide hydrolase to form the corresponding dihydroxyeicosatrienoic acids (DHETs). Epoxidation of arachidonic acid has been attributed to members of the CYP2C (Karara et al., 1993), CYP2E (Laethem et al., 1993), and CYP2J (Wu et al., 1997; Zhang et al., 1997) families, whereas ω-hydroxylation to generate 20-HETE is catalyzed by CYP4A (Nguyen et al., 1999) and CYP4F isoforms (Powell et al., 1998). Although multiple CYP isoforms have been shown in vitro to metabolize arachidonic acid, it is expected that their relative contribution in vivo will be determined by their tissue- and cell-specific patterns of expression and their kinetic characteristics.

The biological functions of the EETs have been extensively studied in isolated cell and organ preparations, yet our understanding of their physiological significance in vivo has been hindered by analytical limitations and the lack of stable and selective EET inhibitors and mimics. EETs are generally considered antihypertensive due to their vasodilatory properties; however, vasoconstrictor effects also have been attributed to the EETs, depending on the vascular bed and species that are studied. Venous or arterial injection of 5,6- and 8,9-EET into rats caused a dose-dependent vasoconstriction and decrease in glomerular filtration rate (Takahashi et al., 1990; Katoh et al., 1991). In both cases, vasoconstriction was dependent on cyclooxygenase activity, suggesting that these EETs are further metabolized by cyclooxygenases to vasocon-

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ABBREVIATIONS: CYP, cytochrome P450; EET, epoxyeicosatrienoic acid; 20-HETE, 20-hydroxyeicosatetraenoic acid; DHET, dihydroxyeicosatrienoic acid; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto; GC-MS, gas chromatography-mass spectrometry; bp, base pair; nt, nucleotide; RT-PCR, reverse transcription-polymerase chain reaction; PFB, pentafluorobenzyl; TMS, trimethylsilyl.

strictive compounds or that they signal the release of vasoactive prostaglandins. In contrast, studies using a rat juxtamedullary nephron preparation characterized 11,12-EET as a potent vasodilator (Imig et al., 1996). This response was stereoselective because 11(R),12(S)-EET but not 11(S),12(R)-EET increased the diameters of the interlobular and afferent arterioles. The function of EETs as endogenous K^+ channel openers provides a potential mechanism for their vasodilatory actions (Hu and Kim, 1993). The inhibitory properties of the EETs toward Na^+-K^+-ATPase and EET-mediated angiotensin II-induced natriuresis also are considered antihypertensive (Hirt et al., 1989; Romero et al., 1991b; Satoh et al., 1993; Ominato et al., 1996). Importantly, the potential renal significance of the CYP-derived eicosanoids in vivo is highlighted by the recent demonstration of endogenous pools of EETs, HETEs, and DHETs in the mouse, rat, and human kidney (Karara et al., 1990; Katoh et al., 1991; Carroll et al., 1997; Ma et al., 1999).

Alterations in renal arachidonic acid metabolism were first implicated in the pathogenesis of hypertension in the spontaneously hypertensive rat (SHR) by Sacerdoti et al. (1988) who showed increased renal microsomal metabolism in SHR relative to normotensive Wistar-Kyoto (WKY) rats. More recently, arachidonic acid ω-hydroxylase activity and CYP4A expression were shown to be increased in the SHR kidney (Omata et al., 1992; Imig et al., 1993; Kroetz et al., 1997) and inhibition of 20-HETE formation in vivo led to a decrease in blood pressure (Su et al., 1998). Support for involvement of the arachidonic acid epoxygenase pathway in the regulation of blood pressure is more limited. Two groups have reported similar epoxygenase activity in WKY and SHR kidneys although chromatographic separation of the DHETs and other eicosanoids was not ideal (Omata et al., 1992; Imig et al., 1993). Studies in Sprague-Dawley rats suggest that a salt-inducible renal epoxygenase has antihypertensive properties (Makita et al., 1994). However, this effect appears to be strain dependent because a high-salt diet decreases renal epoxygenase activity in Dahl salt-sensitive and Brown Norway rats (Ma et al., 1994; Stec et al., 1996) and has no effect in the SHR (Stec et al., 1996). Importantly, CYP-derived eicosanoids also have been implicated in pregnancy-induced hypertension in humans (Catella et al., 1990). Urinary excretion of 8,9- and 11,12-DHET increased in healthy pregnant women compared with nonpregnant controls and excretion of 11,12- and 14,15-DHET increased even further in women with pregnancy-induced hypertension.

Although numerous studies have characterized altered CYP4A expression and 20-HETE formation in the SHR kidney, less information is available on the expression of CYP epoxygenases in this hypertension model. The present study was designed to test the hypothesis that renal CYP epoxygenase activity is altered during the development of hypertension in the SHR. Increased renal CYP epoxygenase activity was found during the developmental phase of hypertension in the SHR and was consistent with increased expression of a novel CYP2J2 immunoreactive protein. This increased epoxygenase activity was specific for the kidney and might represent an important regulatory mechanism for renal EET production. Urinary excretion of EETs also was increased in the SHR, providing evidence for elevated arachidonic acid epoxygenase activity in vivo.

Experimental Procedures

Materials. Radiolabeled arachidonic acid was purchased from Amersham (Arlington Heights, IL) and radiolabeled UTP and CTP from NEN (Boston, MA). Oligonucleotides were synthesized by Operon Technologies, Inc. (Alameda, CA). Restriction enzymes and modifying enzymes were obtained from New England Biolabs (Beverly, MA) or Life Technologies (Gaithersburg, MD). Nitrocellulose membranes were from Micron Separations (Westborough, MA). All molecular biology grade chemicals, HPLC solvents, and ScintiVerse LC were ordered from Fisher Scientific (Pittsburgh, PA). All other reagents were of the highest grade available and were purchased from Fisher Scientific or Sigma Chemical Co. (St. Louis, MO).

Animals. Male SHR and WKY rats were purchased from Charles River Laboratories (Wilmington, MA), housed in a controlled environment with a 12-h light/dark cycle, and fed standard laboratory chow for at least 3 days before use. All animal use was approved by the University of California-San Francisco Committee on Animal Research and followed the National Institutes of Health guidelines for the care and use of laboratory animals. Rats were anesthetized with methoxyflurane, the abdominal cavities were opened, and the kidneys were perfused with ice-cold saline. Perfused kidneys were rapidly removed and dissected into cortex, outer medulla, and inner medulla before immersion in liquid nitrogen. All tissue was stored at −80°C until preparation of RNA or microsomes. In some cases, SHR and WKY rats were housed in metabolic cages for up to 3 days and urine was collected over triphenylphosphine in 24-h intervals. The urine volume was noted and aliquots were stored at −80°C before extraction and quantitation of EETs.

Microsomal Arachidonic Acid Metabolism. Microsomes were prepared from the renal cortex, renal outer medulla, and liver samples from a single animal as described in Kroetz et al. (1997). Microsomal protein concentrations were measured with the Pierce BCA protein assay (Rockford, IL) with BSA as the standard. Renal cortical and hepatic arachidonic acid metabolism was measured in incubations containing [1-^{14}C]arachidonic acid (10 or 85 μM; 0.2 μCi), microsomal protein (0.25 mg/ml), $MgCl_2$ (10 mM), sodium isocitrate (8 mM), and isocitrate dehydrogenase (0.5 I.U.) in potassium phosphate buffer (100 mM; pH 7.4). The mixtures were preincubated for 5 min at 37°C and the reaction was started by addition of NADPH (1 mM). The incubation was continued for 30 min at 37°C and the reaction was terminated by acidifying to pH 3.5 with HCl. Arachidonic acid and its metabolites were extracted twice with ethyl acetate and the combined organic phase was washed once with double distilled water. After evaporation of organic solvent under nitrogen the dry residue was stored at −80°C until HPLC analysis. Metabolites were separated on a 250 × 4.6 mm Alltima C18 5-μm column with an Alltima C18 guard column and in-line filter (Alltech Associates, Deerfield, IL) and eluted at 1 ml/min for 48 min with acetonitrile/water/acetic acid (48:52:0.2%) followed by a linear gradient to acetonitrile/acetic acid (100/0.1%) over 15 min (3.5%/min) (Kroetz et al., 1997). For quantification of the EET regioisomers the elution profile was isocratic flow at 1 ml/min for 52 min with acetonitrile/water/acetic acid (48:52:0.2%), followed by a linear gradient from 48 to 64% acetonitrile over 60 min (0.27%/min), then a linear gradient from 64 to 100% acetonitrile over 10 min (3.6%/min). The identity of the metabolites was previously established by gas chromatography-mass spectrometry (CG-MS) analysis and by coinjection with authentic standards (Kroetz et al., 1997).

RNA Analysis. A full-length CYP2C23 cDNA in pUC19 was kindly provided by Dr. Frank J. Gonzalez (National Cancer Institute, Bethesda, MD). A 333-base pair (bp) *Sac*I/*Xba*I fragment of the CYP2C23 cDNA was ligated into pGEM-7Zf(+) (Promega, Madison, WI), linearized with *Ava*II and transcribed with T7 RNA polymerase to yield a probe of 316 nucleotides (nt) and a protected fragment of 269 nt corresponding to 1416 to 1685 bp of the original cDNA. A fragment of the CYP2E1 cDNA was isolated by standard reverse transcription-polymerase chain reaction (RT-PCR) techniques from

rat liver total RNA with the following primers based on the published cDNA sequence of rat CYP2E1: forward, 5′-GCCACCCTCCTCGTCATATC-3′ and reverse, 5′-GCAGCCAATCAGAAATGTGG-3′ (Song et al., 1986). The isolated fragment spanned the 55- to 534-bp region in the original CYP2E1 cDNA sequence. Total kidney RNA (5 μg) was reverse transcribed using the CYP2E1 reverse primer and Moloney murine leukemia virus reverse transcriptase. An aliquot of the reverse transcription reaction was then used as the template to amplify the desired cDNA fragment. PCR was carried out for 30 cycles with the primers described above and *Taq* DNA polymerase (Perkin Elmer Cetus, Norwalk, CT) under the following conditions: 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, followed by a single final extension at 72°C for 15 min. The 479-bp amplified fragment was digested with *Pvu*I and *Sac*I to give a 330-bp fragment that was ligated into the pSP72 vector (Promega). Digestion of this CYP2E1/pSP72 plasmid with *Ava*II and transcription with T7 RNA polymerase yields a 288-nt probe and a 235-nt protected fragment. A CYP2J3/pCRII plasmid (clone SW9–1) was previously described (Wu et al., 1997). This plasmid was linearized with *Taq*I and transcribed with SP6 RNA polymerase to give a 353-nt probe and a 257-nt protected fragment corresponding to 1521 to 1778 bp of the full-length cDNA. The CYP2J4 cDNA was isolated from rat liver total RNA by RT-PCR with the GeneAmp RNA PCR kit (Perkin Elmer Cetus) and the following two sequence-specific oligonucleotides: 5′-CACCGCGGGCTCTCTGATA-3′ and 5′-CCTTCTCCTCTCACTTGAGCAAG-3′ corresponding to nucleotides 54 to 72 and 1609 to 1631, respectively, of the published CYP2J4 sequence (Zhang et al., 1997). The resulting 1.6-kb PCR product was gel purified with Qiaex gel extraction kit (Qiagen, Valencia, CA) and ligated into the pCRII vector (Invitrogen, Carlsbad, CA). A 396-bp *Eco*RI/*Kpn*I fragment from the CYP2J4/pCRII plasmid was ligated into pGEM-4Z to facilitate transcription. Digestion of this plasmid with *Xmn*I and transcription with SP6 RNA polymerase yields a 305-nt probe and 282-nt protected fragment corresponding to 1349 to 1631 bp of the reported CYP2J4 cDNA sequence. The sequence of all cDNA fragments isolated by RT-PCR was confirmed by DNA sequencing with dideoxy-mediated chain termination and Sequenase 2.0 (United States Biochemical, Cleveland, OH). A rat GAPDH ribonuclease protection probe included as a control in all hybridizations was described previously (Kroetz et al., 1997).

Total RNA was isolated from renal cortex samples by acid/phenol extraction and RNase protection assays were carried out as described for previous studies with the exception that [α-^{32}P]UTP was used in place of CTP for the CYP2C23 and CYP2E1 probes (Kroetz et al., 1997). Gels were dried and protected fragments were visualized with a PhosphorImager and analyzed using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Keeney et al. (1998) recently isolated a novel CYP2J sequence from rat preputial/clitoral gland RNA by RT-PCR. We used a gene specific primer based on this novel sequence (primer 2j10R; 5′-TTCTGGTGACTGTCCATCATACAG-3′) and amplified a 772-bp cDNA fragment from clitoral gland RNA by 5′ rapid amplification of cDNA ends with a kit supplied by Life Technologies. Sequence analysis revealed that this cDNA fragment was ~80% identical with CYP2J3 and CYP2J4. This novel CYP2J sequence has been tentatively designated CYP2J10 by the Committee on Standardized Cytochrome P450 Nomenclature and has been submitted to GenBank (accession number pending). The tissue abundance of CYP2J10 transcripts was determined by RT-PCR with the GeneAmp RNA PCR kit (Perkin Elmer Cetus). The following CYP2J10 gene-specific primers were used: primer 2j10R, 5′-TTCTGGTGACTGTCCATCATACAG-3′; primer 2j10F1, 5′-CGGGCACTGCTTCATTTCAGAAC-3; primer 2j10F2; 5′-TGGAGGCTATCGGAGTGGACAA-3′. Primers 2j10F1 and 2j10R amplified a 764-bp DNA fragment (predicted size) from clitoral gland RNA; primers 2j10F2 and 2j10R amplified a 300-bp fragment (predicted size) from clitoral gland RNA. Reverse transcription was performed with 1 μg of total RNA in a buffer containing 20 mM Tris-HCl (pH 8.3); 50 mM KCl; 2.5 mM $MgCl_2$; 2.5 μM random hexamers; 0.4 mM each of dGTP, dATP, dTTP, and dCTP, and 200 U of Superscript II reverse transcriptase (Life Technologies) incubated at 42°C for 1 h. The PCR amplifications were performed in the presence of 2.5 mM $MgCl_2$, 0.20 μM forward and reverse primers, and 1.0 U of Elongase DNA polymerase (Life Technologies). Following an initial incubation for 150 s at 94°C, samples were subjected to 35 cycles of 45 s at 94°C and 45 s at 54°C and 120 s at 68°C. The PCR products were electrophoresed on 1.0% agarose gels containing ethidium bromide. β-Actin specific primers were used to control for the quality and amount of RNA.

CYP2C24 mRNA levels were detected by Northern hybridization with a 48-mer oligonucleotide from the 3′-untranslated region of the CYP2C24 cDNA (kindly provided by Dr. Jorge H. Capdevila, Vanderbilt University, Nashville, TN). Total cortex RNA (30 μg) was fractionated on a 1% agarose gel containing 2.2 M formaldehyde and transferred to a nylon membrane. The CYP2C24 oligonucleotide or a 20-mer corresponding to nucleotides 230 to 249 of the rat GAPDH cDNA were end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The membranes were hybridized with the radiolabeled probe in QuikHyb solution (Stratagene, LaJolla, CA) for 2 h at 68°C. After hybridization, the membranes were washed twice in 2× standard saline citrate (1× standard saline citrate: 150 mM NaCl, 15 mM sodium citrate, pH 7)/0.1% SDS at 25°C. Bands were visualized with a PhosphorImager and analyzed using ImageQuant software (Molecular Dynamics).

Western Immunoblotting. Renal cortical microsomes (4–45 μg) were separated on an 8% SDS-polyacrylamide gel and transferred to nitrocellulose in 25 mM Tris, 192 mM glycine, and 20% methanol with a semidry transfer system (Bio-Rad). Primary antibodies used in these studies were a rabbit anti-human CYP2J2 IgG (Wu et al., 1996); rabbit anti-rat CYP2C23 antisera, which was a gift from Dr. Jorge H. Capdevila (Vanderbilt University), goat anti-rat CYP2C11 antisera (Gentest Corp., Woburn, MA); rabbit anti-rat CYP2E1 antisera kindly provided by Dr. Kenneth E. Thummel (University of Washington, Seattle, WA); and a rabbit anti-rat CYP2J4 antisera kindly provided by Dr. Laurence S. Kaminsky (Wadsworth Center, Albany, NY). A peptide (QMEHNFMNRPVTLLR) corresponding to amino acids 103 to 117 of the deduced mouse CYP2J9 sequence (W. Qu and D. C. Zeldin, unpublished data) was purchased from Research Genetics (Huntsville, AL). This peptide shares 14 of 15 amino acids with the corresponding region of rat CYP2J3 but only 6 of 15 amino acids with the corresponding region of rat CYP2J4. The peptide was coupled to keyhole limpet hemocyanin via a carboxy-terminal cysteine to enhance antigenicity. The CYP2J9 peptide was then used to generate polyclonal antibodies in New Zealand White rabbits. Western blots were incubated with a 1:500 (CYP2E1)-; 1:3,000 (CYP2J2)-; 1:1,000 (CYP2J4)-; 1:10,000 (CYP2C23)-; 1:1,000 (CYP2C11)-; or 1:2,000 (CYP2J9)-fold dilution of the primary antibody followed by a 1:1,000- to 1:10,000-fold dilution of alkaline phosphatase-conjugated goat anti-rabbit IgG or horseradish peroxidase-conjugated goat anti-rabbit or rabbit anti-goat IgG. Immunoreactive proteins were visualized using an alkaline phosphatase conjugate substrate kit (Bio-Rad) or an enhanced chemiluminescence detection kit (Amersham).

EET Urinary Excretion. Urinary creatinine concentrations were measured by the Medical Center Clinical Laboratories at the University of California-San Francisco. Methods used to quantify endogenous EETs and DHETs present in rat urine were similar to those described by Capdevila et al. (1992). Briefly, urine was collected over triphenylphosphine (5–10 mg), extracted twice under acidic conditions, with two volumes of chloroform/methanol (2:1), and once more with an equal volume of chloroform, and the combined organic phases were evaporated in tubes containing mixtures of [1-^{14}C]8,9-, 11,12-, and 14,15-EET or [1-^{14}C]8,9-, 11,12-, and 14,15-DHET (56–57 μCi/μmol; 30 ng each) internal standards. [1-^{14}C]EET internal standards were synthesized from [1-^{14}C]arachidonic acid (56–57 μCi/μmol) by nonselective epoxidation (Falck et al., 1990). DHET and [1-^{14}C]DHET internal standards were prepared by chem-

ical hydration of EETs and [1-^{14}C]EETs as described by Falck et al. (1990). All synthetic EETs and DHETs were purified by reversed phase HPLC. Saponification to recover phospholipid-bound EETs and DHETs was followed by SiO_2 column purification. The eluent, containing a mixture of radiolabeled internal standards and total endogenous EETs and DHETs, was resolved into individual regioisomers by HPLC as described in Capdevila et al. (1992). EET pentafluorobenzyl (PFB) esters were formed by reaction with pentafluorobenzyl bromide (Karara et al., 1990). Aliquots of individual EET-PFB esters were dissolved in dodecane and analyzed by GC-MS on a VG TRIO-1 quadrupole mass spectrometer (Fisons/VG; Altrincham, Manchester, UK) operating under negative-ion chemical ionization conditions (source temperature, 240°C; ionization potential, 75 eV; filament current, 500 μA) at unit mass resolution, and with methane as a bath gas. Quantifications were made by selected ion monitoring of *m/z* 319 (loss of PFB from endogenous EET-PFB) and *m/z* 321 (loss of PFB from [1-^{14}C]EET-PFB internal standard). The EET-PFB/[1-^{14}C]EET-PFB ratios were calculated from the integrated values of the corresponding ion current intensities. Quantifications of DHETs were made from GC-MS analysis of their PFB esters, trimethylsilyl (TMS) ethers with selected ion monitoring at *m/z* 481 (loss of PFB from endogenous DHET-PFB-TMS) and *m/z* 483 (loss of PFB from [1-^{14}C]DHET-PFB-TMS internal standard). The DHET-PFB-TMS/[1-^{14}C]DHET-PFB-TMS ratios were calculated from the integrated values of the corresponding ion current intensities. Total epoxygenase production was expressed as the sum of EET and DHET excretion and was normalized for kidney function by expressing per 24-h urine volume or creatinine excretion. Control studies demonstrated that under the conditions used, artifactual EET and DHET formation was negligible.

Statistics. All measurements were performed on RNA, protein, or urine samples from individual rats and results are expressed as mean ± S.E. for three to six animals of a given age and strain. Statistical significance of differences between mean values was evaluated by an unpaired Student's *t* test or a one-way ANOVA. Significance was set at a P value of $< .05$.

Results

Renal Arachidonic Acid Epoxygenase Activity. Arachidonic acid metabolism was measured in WKY and SHR renal cortical microsomes and epoxygenase activity was calculated as the sum of EET and DHET formation. Epoxygenase activity was relatively constant throughout the 3- to 13-week period of development in both the WKY and SHR kidney (Fig. 1A). Consistent with previously reported increases in CYP4A activity and 20-HETE formation with age (Omata et al., 1992; Kroetz et al., 1997), the overall contribution of the epoxide pathways to total arachidonic acid metabolism declined steadily from a maximum value of 70% in 3-week-old rats to 39% in 13-week-old rats. With an arachidonic acid concentration of either 10 or 85 μM, epoxygenase activity was increased 25 to 80% in the 4- to 9-week-old SHR microsomes compared with WKY samples. When the formation rates were calculated for the individual EET regioisomers there was a similar increase in 14,15- and 11,12-EET formation in the SHR kidney (1.8- and 1.7-fold, respectively). In contrast, 8,9-EET formation was slightly decreased (28%) in the SHR kidney relative to the WKY kidney (Fig. 1B). There was also a difference in regioselectivity between the WKY and SHR cortex samples. In the WKY, 11,12- and 8,9-EET were formed at 3-fold higher rates than the 14,15-EET regioisomer, whereas SHR cortex microsomes catalyzed the formation of 11,12-EET at roughly twice the rate of the other two regioisomers.

Renal Arachidonic Acid Epoxygenase Protein Expression. The expression of the CYP enzymes that have arachidonic acid epoxygenase activity was characterized to explore the mechanistic basis for the increased activity in the SHR kidney. The protein levels of CYP2C, CYP2E, and CYP2J isoforms were determined in WKY and SHR cortical microsomes by Western blotting. CYP2C23 protein levels were slightly lower in 3- to 5-week-old rats than in older animals and there were no quantitative differences in expression between the WKY and SHR at any age (Fig. 2A). The CYP2C11 antisera detected two immunoreactive protein bands of similar intensity in 3- to 9-week-old rats and only the higher mobility band in 11- to 13-week-old rats (Fig. 2B).

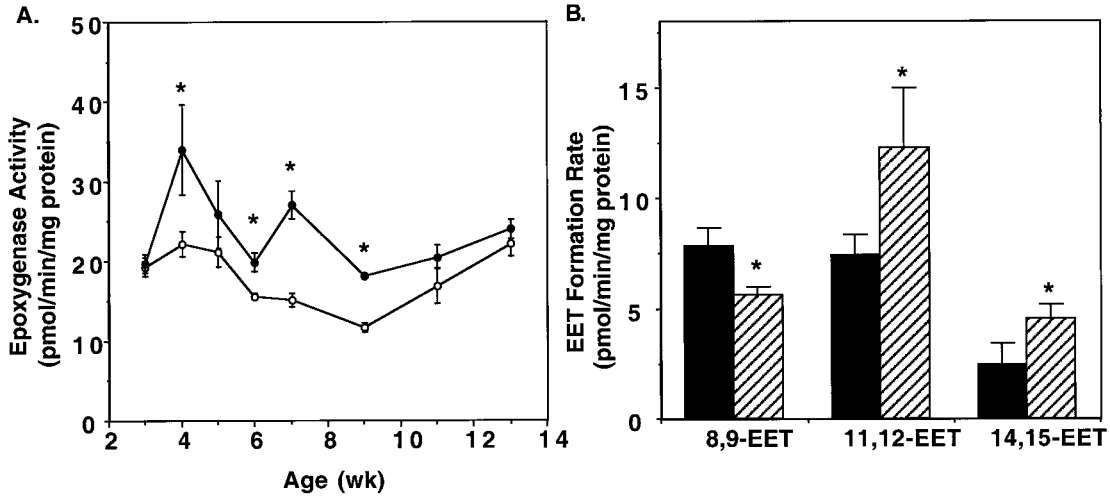

Fig. 1. Renal microsomal arachidonic acid epoxygenase activity in the SHR relative to the WKY rat. A, sum of NADPH-dependent EET and DHET formation in incubations of renal microsomes with [1-^{14}C]arachidonic acid was calculated as total epoxygenase activity. Values are the mean ± S.E. from three to six WKY rats (○) and SHR (●) of a given age. Significant differences between WKY and SHR samples at a given age are indicated ($P <$ .05). B, regioselective EET formation was measured in incubations of arachidonic acid with renal cortical microsomes from 7- to 9-week-old SHR and WKY rats. Values are the mean ± S.E. from six WKY (■) and SHR (▨) samples. Significant differences in EET formation between WKY and SHR microsomes are indicated ($P < .05$). Arachidonic acid epoxygenase activity is increased in 4- to 9-week-old SHR compared with WKY rats and this is accounted for by increased formation of the 14,15- and 11,12-EET regioisomers.

This antisera detects both CYP2C11 and CYP2C13 in male rat liver and the lower mobility band corresponds to CYP2C11 (product information; Gentest Corp., Woburn, MA). Assuming that CYP2C13 also is expressed in the male rat kidney and no additional cross-reactivity with related enzymes, then the lower mobility band is likely to be CYP2C11 and its expression decreases dramatically in older rats. There was no significant difference in expression of either of the CYP2C11 immunoreactive proteins between the WKY and SHR renal cortical microsomes. The expression of CYP2E1 protein was also similar in WKY and SHR cortex during development. CYP2E1 was maximally expressed in the very young animals and decreased significantly in older animals (Fig. 2C). At present, two CYP2J proteins have been identified in the rat, CYP2J3 and CYP2J4 (Wu et al., 1997; Zhang et al., 1997; Scarborough et al., 1999). Western blots with antisera made against recombinant CYP2J4 protein detected a single protein of predicted molecular weight in both WKY and SHR renal cortex. There were no consistent differences in expression of CYP2J4 between the hypertensive and normotensive kidneys (Fig. 2D).

Western blots also were performed with antisera directed against the human CYP2J2 protein that has been shown to cross-react with rat CYP2J proteins but not with members of the rat CYP1A, CYP2A, CYP2B, CYP2C, CYP2E, and CYP4A subfamilies (Wu et al., 1997; Node et al., 1999). CYP2J2 antisera detected two major immunoreactive proteins in rat cortex microsomes (Fig. 2E). In some samples, low levels of at least two other protein bands in the 53- to 58-kDa region were detected. The level of the slower migrating major protein was relatively constant throughout development and there were no differences in expression between the WKY and SHR kidneys. In contrast, the faster migrating major CYP2J2 immunoreactive protein was significantly more abundant at all ages in the SHR compared with the WKY. In the WKY microsomes, the level of the slower migrating protein was more than five times higher than the faster migrating protein, whereas the difference in expression of the two CYP2J2 immunoreactive proteins in the SHR kidneys was generally <3-fold. In fact, there were comparable levels of both major proteins in the 9- and 13-week-old SHR samples. The density of the differentially expressed CYP2J2 immunoreactive protein band was measured in three to four samples for a given strain and age by laser densitometry. The expression of this protein was 1.4- to 3.5-fold higher in the SHR samples compared with WKY samples (data not shown). The increase in CYP2J2 immunoreactive protein is consistent with the increased arachidonic acid epoxygenase activity in the SHR cortex.

In light of the significant difference in CYP2J2 immunoreactive protein expression between WKY and SHR microsomes, it became of interest to identify the specific isoform responsible for the observed pattern of expression. Two lines of evidence suggest that the differentially expressed CYP2J2 immunoreactive protein in SHR kidney is not CYP2J4. First, CYP2J4 protein levels as determined using a polyclonal antibody to the purified CYP2J4 protein were similar in WKY and SHR cortex microsomes (Fig. 2D). Second, the CYP2J4 protein band detected in Fig. 2D comigrated with the upper band detected with the CYP2J2 antisera in Fig. 2E. Further evidence indicates that the differentially expressed CYP2J2 immunoreactive protein is also not CYP2J3. With recombinant CYP2J3 protein expressed in Sf9 cells, it was shown that CYP2J3 migrates slower than both major immunoreactive proteins detected in kidney and liver with the CYP2J2 antisera (Fig. 3). Additional analysis was carried out using a peptide-based antibody to mouse CYP2J9. The 15-amino acid peptide used for the generation of this antisera shared 93% identity with the corresponding region in the rat CYP2J3 protein and only 40% identity with rat CYP2J4. The CYP2J9 antisera recognized the CYP2J3 recombinant protein as a single band of ~57 kDa but failed to detect any immunoreactive proteins in WKY or SHR renal cortex (Fig. 3). In contrast, the CYP2J9 antisera detected very low levels of a 57-kDa immunoreactive protein that comigrated with the CYP2J3 protein in WKY and SHR liver and higher levels of

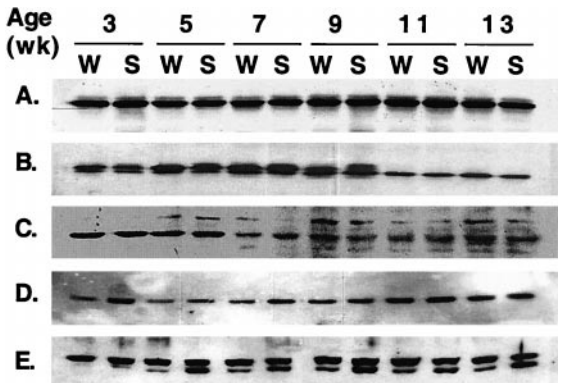

Fig. 2. Western immunoblots of CYP arachidonic acid epoxygenases in WKY and SHR kidney cortex. Microsomal proteins (4–45 μg) from WKY (W) and SHR (S) renal cortex were separated on 8% SDS-polyacrylamide gels, transferred to nitrocellulose, and blotted with antisera against rat CYP2C23 (A), rat CYP2C11 (B), rat CYP2E1 (C), rat CYP2J4 (D), or human CYP2J2 (E). Immunoreactive proteins were detected by alkaline phosphatase staining (CYP2C23) or chemiluminescence (CYP2C11, CYP2E1, CYP2J4, and CYP2J2). The blots shown are representative of results from three to six animals per experimental group. The age of the rats is indicated on the top of the blot. The level of the faster migrating CYP2J2 immunoreactive protein was increased in the SHR kidneys.

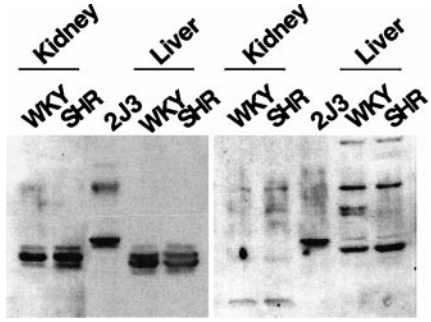

Fig. 3. Immunoblotting of CYP2J proteins in WKY and SHR kidneys and livers. Duplicate samples of WKY and SHR kidney and liver microsomal protein (10 μg) and recombinant CYP2J3 protein (0.5 pmol) were separated on an 8% SDS-polyacrylamide gel and transferred to nitrocellulose. Half of the membrane was blotted with antisera against human CYP2J2 (left) and the other half with mouse CYP2J9 (right). The peptide antigen used for the production of CYP2J9 antisera has 93% identity with the corresponding region of the CYP2J3 protein. Immunoreactive proteins were detected by chemiluminescence and the membranes were realigned to compare the migration of immunoreactive proteins. The CYP2J9 and CYP2J2 antisera recognized the recombinant CYP2J3 protein as a single band of ~57 kDa. No immunoreactive proteins with the same mobility as recombinant CYP2J3 were detected in the liver or kidney with the CYP2J2 antisera. The CYP2J9 antisera detected very low levels of a protein that comigrated with the CYP2J3 standard in WKY and SHR liver but not kidney.

a slightly smaller protein. These data suggest that the differentially expressed CYP2J2 immunoreactive protein is distinct from the two cloned rat CYP2J isoforms.

Increased expression of CYP2J2 immunoreactive protein was not restricted to the cortex because similar changes were found in outer medulla samples from the same animals (Fig. 4A). Expression of the CYP2J2 immunoreactive proteins was significantly less in the outer medulla than in the cortex and was barely detectable in the 3-to 5-week old rats. Two immunoreactive protein bands also were detected in outer medulla samples with the CYP2J2 antisera and in the majority of samples both of these proteins were expressed at significantly higher levels in the SHR. A comparison of Figs. 2E and 4A indicates that the increase in CYP2J2 immunoreactive protein levels was more variable in the outer medulla compared with the cortex, but the general trend of increased expression in the SHR kidney was evident in the majority of the samples that were analyzed.

Renal Arachidonic Acid Epoxygenase mRNA Levels. RNase protection assays were used to quantitate the corresponding levels of CYP2J mRNA in the WKY and SHR cortex. The specificity of each assay was confirmed in preliminary studies with sense RNA transcribed from the full-length cDNAs (data not shown). The expression of both CYP2J3 and CYP2J4 mRNAs was low in the kidney. The level of CYP2J3 mRNA was relatively constant throughout development and CYP2J4 mRNA levels were slightly higher in 7- to 13-week-old animals than in the younger rats. There were no measurable differences in the expression of either of these genes between WKY and SHR kidneys (Fig. 5). These data suggest that the increased level of the CYP2J2 immunoreactive protein in the SHR kidney is not a consequence of increased transcription and/or stabilization of the CYP2J3 or CYP2J4 mRNAs. There was also no evidence of altered expression of CYP2C23, CYP2C24, or CYP2E1 mRNA levels in the SHR kidneys compared with WKY kidneys (data not shown).

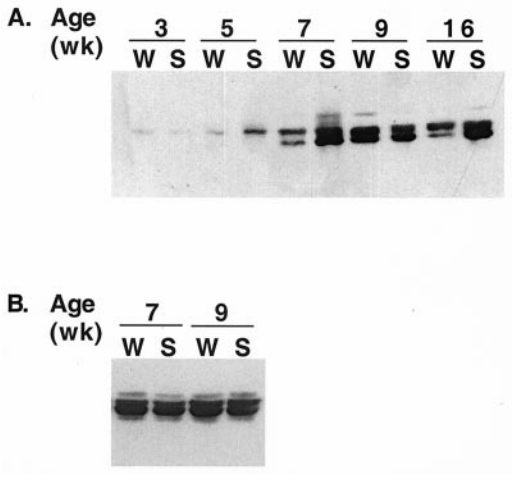

Fig. 4. Expression of CYP2J2 immunoreactive proteins in outer medulla and liver. Western immunoblots were performed on samples from WKY and SHR outer medulla (A) and liver (B). Microsomal proteins (10–15 μg) from WKY rats (W) and SHR (S) were separated on an 8% SDS-polyacrylamide gel, transferred to nitrocellulose, and blotted with antisera against human CYP2J2. Immunoreactive proteins were detected by chemiluminescence. The blots are representative of results from three to six animals per experimental group. The age of the rats is indicated on the top of the blot. The expression of CYP2J2 immunoreactive proteins was increased in the SHR outer medullas but not in the livers.

Recently, Keeney et al. (1998) identified a novel CYP2J sequence (tentatively designated CYP2J10) from rat preputial/clitoral gland RNA. Using 5′ rapid amplification of cDNA ends and a gene-specific primer based on this novel sequence, we amplified a 772-bp CYP2J10 5′-end cDNA fragment from clitoral gland RNA. Based on the sequence of this fragment, we designed a sensitive and specific RT-PCR method to amplify a 764-bp DNA fragment from reverse transcribed rat tail, clitoral gland, and kidney RNA (Fig. 6). The specificity of the primers for CYP2J10 was confirmed in control studies with CYP2J3 and CYP2J4 cDNAs. Densitometry of the amplified CYP2J10 fragment normalized to the β-actin signal revealed that 1) CYP2J10 transcripts were much more abundant (2.5- to 3-fold) in tail and clitoral gland RNA than in kidney RNA; and 2) there was no significant difference in CYP2J10 transcript abundance in kidney RNA from SHR and WKY animals (1.27 ± 0.15 and 1.25 ± 0.22 U, respectively). These results were independently confirmed by amplifying a 300-bp fragment from RNA prepared from these same tissues with a different gene-specific forward primer (Fig. 6). Based on these data, we conclude that CYP2J10 transcripts are present at relatively low levels in the kidney and that SHR and WKY kidneys contain comparable levels of this transcript.

Liver Arachidonic Acid Epoxygenase Activity and Protein Expression. To determine whether the increased CYP epoxygenase activity and CYP2J expression were specific for the kidney, similar measurements were made in WKY and SHR liver microsomal samples. As shown in Fig. 4B, two major CYP2J2 immunoreactive proteins corresponding to those detected in renal cortex microsomes were abundantly expressed in liver microsomes at similar levels in the WKY and SHR. Epoxygenase activity accounted for 50 to 68% of total arachidonic acid metabolism in the livers of 7- and 9-week-old SHR and WKY rats. There were no significant differences in EET formation between the WKY and SHR liver microsomes (Fig. 7).

Urinary Excretion of EETs. The data presented above provides strong in vitro evidence for increased EET formation in the hypertensive rat kidney. Urinary excretion of EETs was measured to evaluate whether this was also apparent in vivo. Urine was collected over a 24-h period in 4- and 8-week-old WKY and SHR rats, and both EETs and DHETs were quantified by GC-MS analysis and used as a measure of total epoxygenase activity. Excretion rates for the

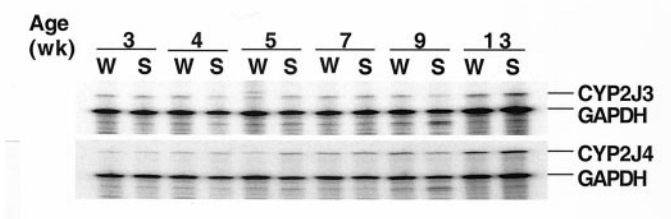

Fig. 5. RNase protection assays of CYP2J epoxygenases in WKY and SHR kidneys. Total cortical RNA was prepared from WKY (W) and SHR (S) kidneys and used for RNase protection assays. RNA was hybridized with a CYP2J3 or CYP2J4 probe and a rat GAPDH probe. The autoradiograms are representative of three to six animals per experimental group. The age of the animals is indicated on the top of the gel. Autoradiograms were visualized with a PhosphorImager and analyzed using ImageQuant software. There were no differences in the mRNA level of either CYP2J3 or CYP2J4 between the WKY and SHR kidneys.

8,9-, 11,12-, and 14,15-EET are shown in Fig. 8. Urinary EET excretion was similar for the 4- and 8-week-old animals and the reported numbers are averages from all samples of a given strain. Similar results were obtained whether excretion rates were corrected for urine volume or for creatinine content. The 14,15-EET regioisomer was the major EET produced in vivo and was excreted at 6- to 14-fold higher rates than the 11,12- or 8,9-EETs. Importantly, in vivo epoxygenase activity expressed as the sum of urinary EET and DHET excretion was significantly increased in SHR compared with WKY kidney, consistent with the increased in vitro EET formation and CYP2J2 immunoreactive protein expression in the SHR kidney. Formation of 14,15-, and 11,12-EET was 2.5- and 1.8-fold higher, respectively, in the SHR compared with WKY rats. The regioselectivity of this effect was also apparent in vivo because urinary excretion of the 8,9-regioisomer was similar in the WKY rats and SHR.

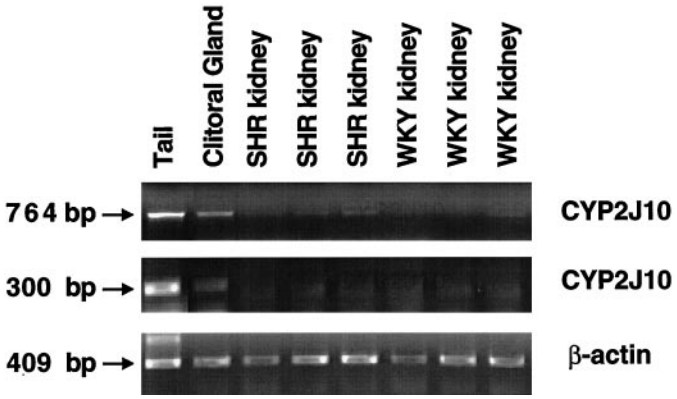

Fig. 6. RT-PCR of CYP2J10 in WKY and SHR kidneys. Total RNA prepared from rat tail, clitoral gland, WKY renal cortex, and SHR renal cortex was reverse transcribed with random hexamers and the product was then amplified by PCR with CYP2J10- or β-actin-specific primers as described in *Experimental Procedures.* An aliquot of the reaction was electrophoresed on a 1% agarose gel and visualized by ethidium bromide staining. One set of CYP2J10-specific primers yielded a product of 764 bp and a second set yielded a product of 300 bp. Prominent bands were amplified with both CYP2J10 primer pairs from rat tail and clitoral gland RNA. In contrast, CYP2J10 mRNAs were barely detectable in the SHR and WKY kidney RNAs.

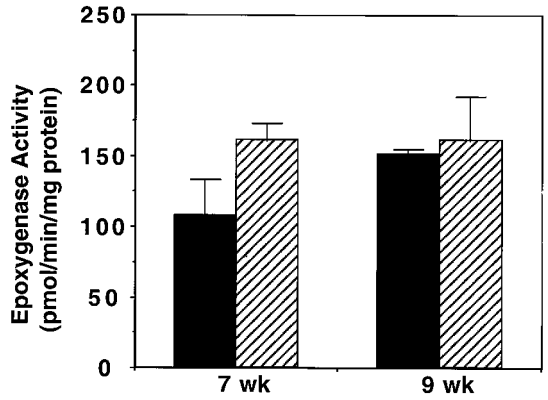

Fig. 7. Hepatic microsomal epoxygenase activity in WKY and SHR kidneys. The sum of NADPH-dependent EET and DHET formation in incubations of hepatic microsomes with [1-^{14}C]arachidonic acid was calculated as total epoxygenase activity. Values are the mean ± S.E. from three to six WKY rats (■) and SHR (▨) of a given age. Arachidonic acid epoxygenase activity was similar in 7- and 9-week-old SHR and WKY rats.

Discussion

Since the first proposal of a role for the altered formation of cytochrome P450-catalyzed arachidonic acid eicosanoids in the pathophysiology of hypertension almost 10 years ago (Sacerdoti et al., 1989), most of the work in this area has focused on the ω-hydroxylase pathway. The present report provides substantial evidence that a component of the renal arachidonic acid epoxygenase pathway is also significantly altered in the SHR model of hypertension. Increased EET formation was found predominantly during the developmental phase of hypertension in the SHR compared with the normotensive WKY strain. This was explained, at least in part, by increased expression of a CYP2J2 immunoreactive protein, whereas changes in expression of other known renal epoxygenases did not occur. Of major significance was the finding of increased urinary EET excretion in the SHR, indicating that increased epoxygenase activity is also present in vivo. The kidney-specific increase in CYP2J2 immunoreactive protein expression raises the possibility that this is one important mechanism for regulating EET formation in the SHR kidney.

Characterization of the biological effects of the EETs has been limited by the lack of selective inhibitors of the CYP epoxygenases, and the absence of stable EET mimics. Furthermore, the hydrolysis of EETs to DHETs, and their further metabolism along other biochemical pathways has not been well characterized in most in vitro and in situ systems used to study EET biological properties, and so it is not clear whether the measured effects are due to the EETs, the DHETs, or their metabolites. As a consequence, both antihypertensive and prohypertensive properties have been attributed to the EETs and their role in vivo is still unclear. The EETs are generally regarded as vasodilatory eicosanoids (Imig et al., 1996) but there is some evidence that they also can act as vasoconstrictors, possibly in a cyclooxygenase-dependent fashion (Takahashi et al., 1990; Katoh et al., 1991; Imig et al., 1996). This suggests that increased EET formation in the SHR kidney could lead to either vasodilation or vasoconstriction. The observed effect will be largely depen-

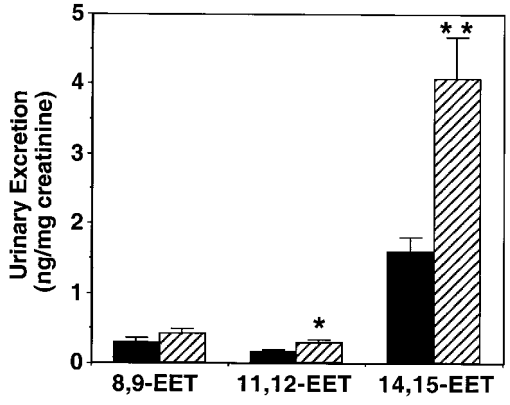

Fig. 8. Urinary EET excretion in WKY rats and SHR. Urine was collected for 24 h from untreated WKY rats (■) and SHR (▨). EETs and DHETs were extracted from urine and quantified by GC-MS as described in *Experimental Procedures.* The values shown (mean ± S.E. of four animals per strain) are the sum of EET and DHET excretion and represent in vivo epoxygenase activity. Significant differences between SHR and WKY are indicated (**$P < .01$; *$P < .05$). The in vivo production of 14,15- and 11,12-EET was increased 2.5- and 1.8-fold, respectively, in the SHR relative to the WKY rat.

dent on the ratio of vasodilatory EETs (mainly 11,12- and 14,15-EET) to vasoconstrictive EETs (primarily 5,6-EET). The 5,6-EET metabolite is extremely labile, making its formation difficult to measure in vitro and in vivo. The rat renal epoxygenases, CYP2C23, CYP2C11, CYP2C24, and CYP2J3, produce negligible amounts of 5,6-EET, and only minor amounts of this epoxide are formed in Sprague-Dawley kidney microsomes (Capdevila et al., 1992; Karara et al., 1993; Wu et al., 1997; Holla et al., 1999). In vivo, the 14,15-, 11,12-, and 8,9-EETs are all formed at measurable levels, with the 14,15-EET being the most abundant regioisomer excreted in rat urine. Effects of the EETs on renal tubular ion and water transport also have been described. Potent inhibition of the Na^+-K^+-ATPase pump by 5,6- and 11,12-EET would lead to an increased Na^+ and water excretion (Satoh et al., 1993), buffering the deleterious effects of increased blood pressure. Natriuresis also is mediated by 5,6-EET in an angiotensin II-induced manner (Romero et al., 1991a). Although 8,9- and 14,15-EET activate Na^+/H^+ exchange in rat glomerular mesangial cells (Harris et al., 1990), a similar effect has not been demonstrated in renal epithelial cells and it is not clear if such a mechanism is involved in the regulation of natriuresis.

The increased EET formation in the SHR kidney is consistent with increased expression of an unknown CYP2J2 immunoreactive protein. Until recently CYP2C isoforms were considered the major renal arachidonic acid epoxygenases. In particular, in the rat kidney CYP2C23 is highly expressed and catalyzes the formation of 8,9-, 11,12-, and 14,15-EET in roughly a 1:2:1 ratio (Karara et al., 1993). An important role for this isoform in the regulation of renal EET formation is suggested by the recent evidence for induction of CYP2C23 by excess dietary salt intake (Holla et al., 1999). The CYP2C11 and CYP2C24 isoforms are expressed in the rat kidney at much lower levels than CYP2C23, and their pattern of regioselectivity for EET formation is not consistent with a major role for these isoforms in vivo (Holla et al., 1999). CYP2E1 also has arachidonic acid epoxygenase activity although it is much more efficient in subterminal hydroxylation than in epoxidation, and the contribution of this enzyme to EET formation in vivo is likely to be minor (Laethem et al., 1993). In the present study, CYP2C23, CYP2C11, CYP2C24, and CYP2E1 were expressed at similar levels in the WKY and SHR kidney cortex and could not account for the measured differences in arachidonic acid epoxidation.

In the past several years, multiple CYP2J enzymes have been isolated and characterized as arachidonic acid epoxygenases (Wu et al., 1996, 1997; Zhang et al., 1997; Ma et al., 1999; Scarborough et al., 1999). At the present time, two rat CYP2J proteins have been identified, CYP2J3 and CYP2J4. Western blots developed with human CYP2J2 antisera detected two distinct immunoreactive proteins in the WKY and SHR kidneys. Additional blots with antisera made against recombinant CYP2J4 detected only a single protein in kidney microsomes that was previously shown to comigrate with purified CYP2J4 protein (Zhang et al., 1998) and that was not differentially expressed in SHR and WKY animals. A comparison of Western blots developed with CYP2J4 and CYP2J2 antisera suggests that the upper band detected with the latter antisera represents the CYP2J4 protein, although positive identification of this band awaits the availability of recombinant CYP2J4 protein standard. Our data indicate that both of the major proteins detected with the CYP2J2 antisera migrate at a faster rate than recombinant CYP2J3 and that CYP2J3 protein levels are very low in the kidney. Collectively, these findings suggest that neither CYP2J3 nor CYP2J4 is differentially expressed in the hypertensive rat kidney. The possibility that this differentially expressed protein in the SHR is not a member of the CYP2J family cannot be completely ruled out, although previous characterization of the CYP2J2 antisera failed to reveal significant cross-reactivity with other CYP proteins, including members of the CYP1A, CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, and CYP4A subfamilies (Wu et al., 1996, 1997; Node et al., 1999). At least six distinct CYP2J genes have been identified in the mouse (J. Ma and D. C. Zeldin, unpublished data) and it is reasonable to expect that additional members of the rat CYP2J family have yet to be identified (Scarborough et al., 1999). In this regard, a partial cDNA with ~80% identity to CYP2J3 and CYP2J4 was recently isolated from rat preputial/clitoral gland RNA (Keeney et al., 1998). However, our preliminary RT-PCR results indicate that expression of this gene is very low in both the WKY and SHR kidney. Although our RT-PCR reactions were not designed to be quantitative there was no apparent difference in the level of CYP2J10 mRNA in the WKY and SHR kidneys, suggesting that this is not the differentially expressed CYP2J2 immunoreactive protein in the SHR.

A comparison of in vivo epoxygenase activity measured as urinary EET and DHET excretion in WKY and SHR is consistent with the in vitro evidence for increased renal microsomal epoxygenase activity and induction of a CYP2J2 immunoreactive protein in the SHR kidney. The fact that 14,15-EET excretion was increased in the SHR relative to the WKY rat to a larger extent than the other regioisomers is consistent with an induction of a CYP2J epoxygenase. The rat CYP2J3, human CYP2J2, and mouse CYP2J5 isoforms all preferentially catalyze epoxidation of arachidonic acid at the 14,15-position (Wu et al., 1996, 1997; Ma et al., 1999). There was a good correlation between in vitro and in vivo EET formation for each of the regioisomers, with both 14,15- and 11,12-epoxygenase activity being significantly elevated in the SHR kidney. In contrast, 8,9-EET formation was similar in the WKY rat and SHR when measured both in vitro and in vivo, illustrating the regioselectivity of this effect.

Numerous studies have reported differences in renal CYP arachidonic acid metabolism in the SHR versus WKY kidney, although the specific alterations and their magnitude varies significantly across the studies (Sacerdoti et al., 1988; Omata et al., 1992; Imig et al., 1993; Kroetz et al., 1997). In contrast, only a few studies have looked at urinary excretion of the CYP-derived eicosanoids in rat hypertension models (Schwartzman et al., 1991; Capdevila et al., 1992. The present results provide the first in vivo evidence for increased EET formation in the SHR relative to the normotensive WKY rat. Several explanations for the role of this increased EET formation in the hypertensive phenotype can be proposed. As mentioned above, both prohypertensive and antihypertensive properties have been attributed to the EETs (Takahashi et al., 1990; Katoh et al., 1991; Satoh et al., 1993; Imig et al., 1996). It is possible that the prohypertensive properties of the EETs predominate in the SHR kidney, leading to cell-specific effects on renal tubular sodium transport and vascular tone that result in Na^+ retention and/or

vasoconstriction. Alternatively, the residence time of the EETs in the kidney may be short as a result of their hydrolysis to the corresponding DHETs. Little attention has been directed at the biological effects of the DHETs on renal function and vascular tone although, in general, it is assumed that DHET formation eliminates and or attenuates the effects of the EETs. The possibility that inherent biological properties of the DHETs mask the effects of the EETs has yet to be explored. A final explanation is that increased EET formation is a consequence of rather than a cause for the elevated blood pressure. This is consistent with the protective properties of the EETs in other model systems (Wu et al., 1997) and might represent a compensatory response of the SHR to deleterious increases in blood pressure. The role of CYP-mediated eicosanoid formation in the regulation of renal function and vascular tone is increasingly recognized as complex and multifactorial. Increased expression of CYP4A3 and renal microsomal 20-HETE formation have previously been documented in the SHR kidney (Kroetz et al., 1997). Parallel increases in EET formation might mitigate, at least in part, the vasoconstrictive effects associated with elevated 20-HETE levels.

In summary, we have described increased expression of a CYP2J2 immunoreactive protein in the SHR kidney that is associated with increased EET formation both in vitro and in vivo. Several lines of evidence suggest that the differentially expressed CYP2J2 immunoreactive protein is novel and this will be isolated, cloned, and characterized in future studies. Increased EET formation in the SHR is consistent with either a previously unrecognized prohypertensive role for the EETs or a compensatory response to elevated blood pressure, and illustrates the complex nature of the renal CYP eicosanoid system. A complete understanding of the role of CYP eicosanoids in blood pressure regulation awaits the availability of potent and selective epoxygenase and ω-hydroxylase inhibitors and stable EET and 20-HETE mimics that can be used in vivo.

Acknowledgments

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